PATENT COOPERATION T EATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/040389	03.12.2004	05.12.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
ERASMUS MC

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Schmitt, C

Telephone No. +49 89 2399-7351

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/040389

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/040389

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ The International Searching Authority has not been able to consider the validity of the priority claim because a copy of the earlier application whose priority has been claimed was not available to the International Searching Authority at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/040389

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-18

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 1-7, 9-18 (all partially), 8 (completely)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/040389

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	17,18
	No:	Claims	1-7,9-16
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-7,9-18
Industrial applicability (IA)	Yes:	Claims	1-7,9-18
	No:	Claims	none

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: WO/02068579

D2: "Affymetrix GeneChip Human Genome U95 Set HG-U95A" GENBANK GEO, 11 March 2002 (2002-03-11), XP002293987.

D3: PAIK S ET AL: "MULTI-GENE RT-PCR ASSAY FOR PREDICTING RECURRENCE IN NODE NEGATIVE BREAST CANCER PATIENTS-NSABP STUDIES B-20 AND B-14" BREAST CANCER RESEARCH AND TREATMENT, NIJHOFF, BOSTON, US, vol. 82, no. SUPPL 1, 3 December 2003 (2003-12-03), pages S10-S11,ABSTRN, XP001202622 ISSN: 0167-6806.

D4: JANSEN M ET AL: "Molecular classification of tamoxifen-responsive and -resistant breast carcinomas by gene expression profiling." BREAST CANCER RESEARCH AND TREATMENT, vol. 82, no. Supplement 1, November 2003 (2003-11), page S14, XP002321901 & 26TH ANNUAL SAN ANTONIO BREAST CANCER SYMPOSIUM; SAN ANTONIO, TX, USA; DECEMBER 03-06, 2003 ISSN: 0167-6806

D5: STREEKUMAR A ET AL: "USING PROTEIN MICROARRAYS TO STUDY CANCER" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, no. SUPPL, December 2002 (2002-12), pages 46-53, XP009018820 ISSN: 0736-6205

D6: LOS GERRIT ET AL: "Using mRNA expression profiling to determine anticancer drug efficacy" CYTOMETRY, vol. 47, no. 1, 1 January 2002 (2002-01-01), pages 66-71, XP002321902 ISSN: 0196-4763

D7: DOWSETT MITCH: "Biomarker investigations from the ATAC trial: the role of TA01." BREAST CANCER RESEARCH AND TREATMENT. 2004, vol. 87 Suppl 1, 2004, pages S11-S18, XP002321903 ISSN: 0167-6806

D8: MA X-J ET AL: "A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen" CANCER CELL, XX, US, vol. 5, no. 6, June

2004 (2004-06), pages 607-616, XP002317299 ISSN: 1535-6108

D9: JANSEN MAURICE P H M ET AL: "Molecular classification of tamoxifen-resistant breast carcinomas by gene expression profiling." JOURNAL OF CLINICAL ONCOLOGY : OFFICIAL JOURNAL OF THE AMERICAN SOCIETY OF CLINICAL ONCOLOGY. 1 FEB 2005, vol. 23, no. 4, 1 February 2005 (2005-02-01), pages 732-740, XP009045184 ISSN: 0732-183X

D10: DATABASE EMBL [Online] 3 February 2004 (2004-02-03), "Sequence 3748 from Patent WO02068579." XP002321904 retrieved from EBI accession no. EM_PAT:CQ717814 Database accession no. CQ717814

D11: DATABASE EMBL [Online] 3 February 2004 (2004-02-03), "Sequence 15057 from Patent WO02068579." XP002321905 retrieved from EBI accession no. EM_PAT:CQ729123 Database accession no. CQ729123

D12: DATABASE EMBL [Online] 3 February 2004 (2004-02-03), "Sequence 10220 from Patent WO02068579." XP002321906 retrieved from EBI accession no. EM_PAT:CQ724286 Database accession no. CQ724286

D13: DATABASE EMBL [Online] 3 February 2004 (2004-02-03), "Sequence 4515 from Patent WO02068579." XP002321907 retrieved from EBI accession no. EM_PAT:CQ718581 Database accession no. CQ718581

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Independent claim 1 relate to a set of marker genes comprising two or more genes identified in Table 1 as differentially expressed in primary tumors of recurring breast cancer patients exhibiting a outcome to anti-estrogen therapy.
However, no gene is identified in Table 1. In view of the description (e.g. page 7, 2nd

paragraph), which indicates that the listing of genes in the 81 gene predictive signature is found in Figure 2, and the fact that Figure 2A and Figure 2B are listing the same 81 genes; claim 1 is interpreted as relating to a set of marker genes comprising two or more genes identified in Figure 2B as differentially expressed in primary tumors of recurring breast cancer patients exhibiting a outcome to anti-estrogen therapy.

Due to the expression "two or more genes identified in Figure 2B", claim 1 relates to an exceedingly number of possible combination of marker genes. The claim contains so many possible permutations that a lack of clarity and conciseness within the meaning of Article 6 PCT arises to such an extent that a meaningful search is only possible for particular combinations of genes that are clear and credibly supported and disclosed in the present application.

In view of the description (page 6, page 7, paragraphs 2-4, and page 20, last paragraph-page 21, first paragraph) and present claims 2-8, the only such sets of marker genes are a set comprising the 81 genes of the 81-gene signature listed in Figure 2B, the 44 genes of the 44-gene signature listed in Figure 2B, and the combination of one or both of DEME-6 and CASP2 with one or both of SIAH-2 and TNC.

However, it appears that at least one of the genes listed in Figure 2B is not clearly defined (Article 6 PCT). In particular, one of the genes listed in Figure 2B is only identified by its unigene accession number Hs.437986. Said unigene accession number has however been deleted from the database. The gene identified by the unigene accession number Hs.437986 belongs to both 81- and 44-gene signatures.

2. The search of claim 1 has therefore been limited to a set of marker genes comprising one or both of DEME-6 and CASP2, and one or both of SIAH-2 and TNC identified in Figure 2B as differentially expressed in primary tumors of recurring breast cancer patients exhibiting a outcome to anti-estrogen therapy, with a significance of $p<0.05$ (i.e. the subject-matter of claim 7).

The search of dependent claims 2-7 has been restricted accordingly.

3. Similarly, the search of claims 9-18, which have a back reference to claims 1-7, has been restricted accordingly.

In particular, the search of claim 9 has been restricted to a nucleic acid probe comprising a marker gene selected from DEME-6, CASP2, SIAH-2 and TNC.

4. Furthermore, independent claim 15 relates to an assay system for diagnosing patient response to anti-estrogen therapy for recurring breast cancer, comprising binding ligands that specifically detect polypeptide encoded by each of the respective marker genes of the set of marker genes comprising one or both of DEME-6 and CASP2, and one or both of SIAH-2 and TNC.
The functional feature "binding ligands" however does not allow to deduce the structural feature(s) characterising said "binding ligands", so that the exact binding ligands involved remain unclear. These unclear "binding ligands" leave therefore doubts as to the subject-matter encompassed by claim 15. Claim 15 lacks therefore clarity (Article 6 PCT) to such an extent as to render a meaningful search impossible over the whole claimed scope.
The only clear "binding ligands" identified in the present application (i.e. claim 16) are antibodies or binding fragment thereof.
The search of claim 15 has therefore been limited to "binding ligands" being antibodies or binding fragment thereof (i.e. subject-matter of claim 16).

5. As claims, or parts of claims, relating to subject-matter in respect to which no international search has been performed need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT), an opinion of the international searching authority will only be given with respect to the subject-matter of the claims that was searched.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Document D1 (the references in parentheses applying to this document) discloses nucleic acid arrays comprised of polynucleotides based on the transcript sequences selected from the sequences SEQ ID NO:1-39010, wherein the sequence of SEQ ID NO: 4515 is the CASP2 transcript sequence, the sequence of SEQ ID NO: 10220 is

the TNC transcript sequence, the sequence of SEQ ID NO: 15057 is the DEME-6 transcript sequence and the sequence of SEQ ID NO: 3748 is the SIAH-2 transcript sequence, or a portion thereof (page 12, lines 18-34 and claims 4, 8, 12 and 16).
The nucleic acid arrays disclosed in document D1 are suitable for diagnosing patient response to anti-estrogen therapy for recurring breast cancer. The subject-matter of independent claim 11 and dependent claims 12-14 is therefore anticipated by document D1.
Claims 11-14 are therefore not new in the sense of Article 33(2) PCT.

2. Furthermore, isolated fragments comprising the exon and/or transcript sequences provided in SEQ ID NO:1-39010, and fragment thereof, are used as probes/primers for detecting gene expression (page 15, lines 23-31).
Claims 9 and 10 are therefore not new in the sense of Article 33(2) PCT.

3. In addition, taking into account that the arrays disclosed in document D1 are considered a set of marker genes comprising one or both of DEME-6 and CASP2, and one or both of SIAH-2 and TNC, claims 1-7 are not new in the sense of Article 33(2) PCT.

It is pointed out that the fact that CASP2, DEME-6, SIAH-2 and TNC are differentially expressed in primary tumors of recurring breast cancer patients exhibiting a outcome to anti-estrogen therapy is not seen as a limiting feature of the set of marker genes claimed in claims 1-7.

4. Document D1 also discloses detection kits, such as, but not limited to, arrays, Taqman probe/primer sets, comprising detection elements, such as nucleic acid probes, that are based on the transcript sequences of SEQ ID NO: 1-39010 (page 20, lines 5-9). Other detection kits may be based on blotting techniques such as northern blots (for detecting RNA), southern blots (for detecting DNA), or western blots (for detecting proteins) (page 24, lines 3-7).
As the technique of western blot implies that antibodies specific for the protein to be detected are used, a kit based on western blot for detecting the protein encoded by SEQ ID Nos: 1-39010, is considered as an assay system suitable for diagnosing patient response to anti-estrogen therapy for recurring breast cancer as defined by

claims 15 and 16.
Claims 15 and 16 are therefore not new in the sense of Article 33(2) PCT.

5. Similarly, the subject-matter of claims 1-7 and 11-14 is also considered to be anticipated by the disclosure of document D2; i.e. the human Genome U95A affymetrix GeneChip (see cited passages in the International Search Report).

6. Document D4 discloses the use of gene expression profiling to discriminate between breast cancer patients with an objective response to tamoxifen or patients with progressive disease. A set of 143 genes, optimized to 40 genes, that is differentially expressed between tamoxifen-responsive and -resistant breast tumors has been identified and is used to predict the outcome of response to tamoxifen. This set includes genes associated with cell proliferation, signal transduction, RNA and/or DNA binding activity, extracellular matrix and protein serine-threonine kinases.

Claim 17 differs from the disclosure of document D3 in that the set of marker genes used to predict the outcome of anti-estrogen therapy for recurring breast cancer is a set of marker genes comprising one or both of DEME-6 and CASP2, and one or both of SIAH-2 and TNC.

Claim 17 is therefore new in the sense of Article 33(2) PCT.

In view of document D4, the problem to be solved by independent claim 17 may be seen as an attempt to provide an alternative method for predicting outcome of anti-estrogen therapy for recurring breast cancer.

The alleged solution provided by claim 17 is to analyse a patient's primary tumor tissue for the expression of one or both of the marker genes DEME-6 and CASP2, and one or both of the marker genes SIAH-2 and TNC; and correlate a cluster 1 expression pattern of said marker genes with prediction of progressive disease; and correlating a cluster 2 expression pattern of said marker genes with prediction of objective response to anti-estrogen therapy.

The applicant has only shown that the genes DEME-6, CASP2, SIAH-2 and TNC

were differentially expressed in primary breast tumor resistant to tamoxifen therapy and those which are sensitive to tamoxifen therapy and that DEME-6, CASP2 and SIAH-2 are individual markers for predicting tamoxifen resistance (i.e. progressive disease).
However, taking into account the fact that various endocrine therapies have different antitumor efficacy (see document D9, page 739, col.2, last paragraph) or the fact that anticancer drugs having different mechanism of action have their own unique expression profile (see document D3, page 69, col.1, 2nd paragraph-col.2, first paragraph); it is not possible to the skilled person, apart from tamoxifen response, to predict the outcome to another anti-estrogen therapy based on the differential expression of the above genes as there is no support in the present application as to the fact that said genes could serve as a signature for predicting outcome of other anti-estrogen therapy. This is moreover true in view of the disclosure of the postpublished document D9 (page 739, col.2, last paragraph), which is from the same applicant as the present application, indicating that future studies will determine whether the proposed profile is able to predict failure of treatment with other endocrine agents as tamoxifen.
Furthermore, in the absence of clear definition of the cluster 1 expression pattern and cluster 2 expression pattern (i.e. which genes are overexpressed/underexpressed in case of progressive disease/objective response), by analyzing the expression of one or both of the marker genes DEME-6 and CASP2, and one or both of the marker genes SIAH-2 and TNC, the skilled person will not be able to correlate this expression of genes with progressive disease or with objective response to tamoxifen therapy as the skilled person does not know which pattern of expression is associated with objective response and which is associated with progressive disease.
Thus, claim 17, does not appear to solve the aforedefined problem. Claim 17 is, therefore, considered to lack an inventive step in the sense of Article 33(3) PCT.

7. A similar reasoning applies to claim 18. Thus, claim 18 is considered to lack an inventive step in the sense of Article 33(3) PCT.

Re Item VI
Certain documents cited

It is assumed that the present claims 1-18 validly claim the priority date of 5.12.2003. Documents D6 and D7 cited as P documents in the International Search Report are not relevant at present.

Should the claimed priority be invalid, then documents D6 and D7 could become relevant to the question of novelty and/or inventive step of claims 9-18.

Re Item VIII
Certain observations on the international application

1. Claim 9 lacks clarity in the meaning of Article 6 PCT due to the expression "comprising at least 10-50 contiguous **nucleic acids**".

2. Furthermore, the expression "cluster 1 expression pattern" and "cluster 2 expression pattern" used in claims 17 and 18 are unclear in the sense of Article 6 PCT. In view of the description (page 4, paragraph 4), cluster 1 is the gene expression profile shown in Figure 2A as correlating with progressive disease, whereas cluster 2 is the gene expression profile shown in figure 2A as correlating with objective response. However, from figure 2A, it is not possible to unambiguously derive the gene expression profile correlating with progressive disease and that correlating with objective response. Moreover, the clustering of gene expression is dependent on the algorithms used for the clustering and expression analysis. Therefore, even if the gene expression patterns correlating with objective response/progressive disease could be derived from Figure 2, said expression pattern could only be assessed using the same algorithms as used in the present application.
Claims 17 and 18 are therefore not supported in the sense of Article 6 PCT and the application does not fulfill the requirements of Article 5 PCT.